Journal of Academia and Industrial Research (JAIR)
Volume 5, Issue 9 February 2017

ISSN: 2278-5213

REVIEW

Punica granatum: A Review on Phytochemicals, Antioxidant and Antimicrobial Properties

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Abstract

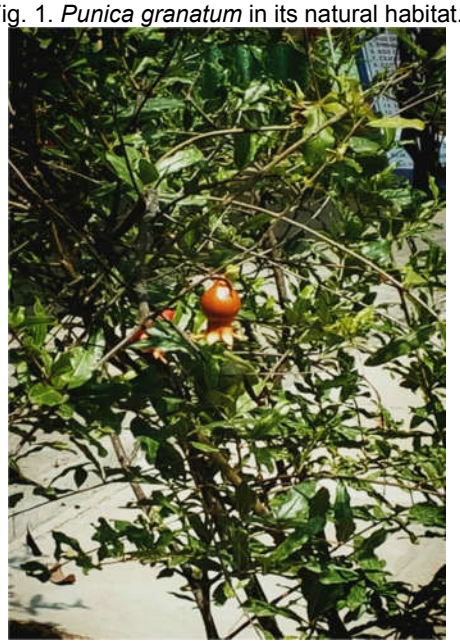
Our world is rich in herbal plants and it is impossible for mankind to survive without the help of plants, every product consists of herbs and every plant has some medicinal effects. In recent years, intensive research has been carried out either to characterize some amazing properties of plant materials to isolate and identify the compounds responsible for those activities. The practice of Ayurvedic medicine has alleviated illnesses and attributed overall positive health for many years. The Indian subcontinent has a rich flora of various plants used in traditional medical treatments. These plants contain different bioactive ingredients used to cure diseases or relieve pain. Pomegranate (*Punica granatum* L., Punicaceae) is an ancient, beloved plant and fruit. The name ‘‘pomegranate’’ follows the Latin name of the fruit *Malum granatum*, which means ‘‘grainy apple.’’ The generic name Punica refers to Pheonicia (Carthage) as a result of mistaken assumption regarding its origin. The pomegranate and its usage are deeply embedded in human history, and utilization is found in many ancient human cultures as food and as a medical remedy. Despite this fact, pomegranate culture has always been restricted and generally considered as a minor crop. The pomegranate tree requires a long, hot and dry season in order to produce good yield of high-quality fruit. Pomegranates are native to central Asia, but since the pomegranate tree is highly adaptive to a wide range of climates and soil conditions, it is grown in many different geographical regions including the Mediterranean basin, Asia, and California. Recent scientific findings corroborate traditional usage of the pomegranate as a medical remedy and indicate that pomegranate tissues of the fruit, flowers, bark, and leaves contain bioactive phytochemicals that are antimicrobial, reduce blood pressure, and act against serious diseases such as diabetes and cancer. These findings have led to a higher awareness of the public to the benefits of the pomegranate fruit, particularly in the western world, and consequently to a prominent increase in the consumption of its fruit and juice. This review provides information about its phytochemicals, antioxidant and antimicrobial properties.

Keywords: Pomegranate, *Punica granatum*, phytochemicals, antioxidant, antimicrobial.

Introduction

Pomegranate, *Punica granatum* L., is a fruit tree well-known by ancient civilisations and lauded because of its medicinal properties (Fig. 1). Pomegranate is considered one of the oldest known edible fruit that is mentioned in the Bible, the Koran, the Jewish Torah, and the Babylonian Talmud as ‘Food of Gods’ where is symbolic of abundance, fertility and prosperity (Aviram *et al.*, 2000; Seeram *et al.*, 2006). It was also mentioned in ancient Greek mythology and Chinese alchemical adepts. For thousands of years, many cultures have believed that pomegranate have beneficial effects on health, fertility, longevity and rebirth (Dahham *et al.*, 2010). The pomegranate is the predominant member of two species comprising the Punicaceae family. The genus name *Punica*, was the Roman name for Carthage, where the best pomegranates were known to grow. Pomegranate literally translates to seeded ("granatus") apple ("ponium") (Jurenka, 2008). This tree is native of the Middle East, Centre of origin IV of Vavilov, which includes interior of Asia Minor, all of Transcaucasia, Iran, and the highlands of Turkmenistan (Vavilov, 1951).

Fig. 1. *Punica granatum* in its natural habitat.



Systematic of pomegranate:

Division	Angiospermae
Class	Magnoliopsida
Subclass	Rosidae
Order	Myrtales
Family	Punicaceae
Genus	*Punica*
Species	*granatum*

Phytochemicals of *Punica granatum*

Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plants that manufacture them may have little need for them. They are naturally synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds, etc. i.e. any part of the plant body may contain active components. The quantity and quality of phytochemicals present in plant parts may differ from one part to another. Moreover, plant secondary metabolites present chemical and pharmaceutical properties interesting for human health (Raskin *et al.,* 2002; Reddy *et al.,* 2003). Compounds belonging to the terpenoids, alkaloids and flavonoids are currently used as drugs or as dietary supplements to cure or prevent various diseases (Raskin*et al.,* 2002) and in particular some of these compounds seem to be efficient in preventing and inhibiting various types of cancer (Watson *et al.,* 2001; Reddy *et al.,* 2003). Pomegranate is an amazing source of cyaniding, delphinidin (both are anthocyanidins), caffeic acid, chlorogenic acid (both are phenolic acids), gallic acid, ellagic acid (tannic acids), luteolin, quercetin (flavones), kaempferol (a flavonol), naringenin (a flavanone) as well as 17 alphaestradiol, estrone, estriol, testosterone, betasistosterol, coumesterol, gamma-tocopherol, punicie acid, campesterol and stigmasterol in its juice, peels and seed oil that are chemopreventive and therapeutic potentials of this plant (Kim *et al.,* 2002; Lansky *et al.,* 2007).

Yasoubi *et al.* (2007) evaluated peel extract which contain substantial amounts of polyphenols such as ellagic tannins, ellagic acid and gallic acid. It has been used in the preparation of tinctures, cosmetic, therapeutic formula and food recipes and in this regard pomegranate peel is a good source of antioxidants. Several extraction techniques have been reported for the extraction of phenolic compounds from different matrices using solvents with different polarities, such as methanol, water, ethyl acetate and petroleum ether. Furthermore, supercritical CO_2 and solvent extraction along sonication have been applied for this purpose. The aim of their research was to compare solvent extraction (acetone, methanol, ethanol, water and ethyl acetate) with and without Sonication and with the SFE (supercritical fluid extraction) process.Furthermore, in this study, the effect of concentrated pomegranate peel extracts (PPEs) on the stability of soybean oil during heating has been compared with that of two synthetic antioxidants BHA and BHT. Gallic acid, granatine A, corilagine and ellagic acid have been isolated from the pericarp the fruit contains an ellagitanin and ellagicacid.

Fredes (2014) studied high variability of analytical method that is used to quantify the phenolic content and that is associated with sample extraction has been reported; therefore, a valid comparison among species and varieties is not always performed correctly. To perform a valid comparison of the phenolic levels in fruits and their derived products, it is necessary to use the same method of extraction and a comparative methodology for polyphenols and antioxidant activity quantification. The objective of their study was to compare the total phenolic and anthocyanin contents and antioxidant activity (FRAP and radical DPPH methods) of polyphenol rich fruit species that using the same extraction and analytical methods. Polyphenolic compounds consist of different phenolic rings, out of which one of the major subgroups of these secondary metabolites are flavonoids. They show some functionality in the plant related to interaction with environment such as plant protection against ultraviolet radiation and antimicrobial properties to protect plants against micro organisms. As human consumption aspects, flavonoids are one of the major groups of phytochemicals with high antioxidant activity. It has been acknowledged that phenolic compounds such as flavonoids and anthocyanins are the major class of effective antioxidants in many fruits and vegetables. Antioxidant activity, total phenolic and flavonoid contents of 9 different Iranian pomegranate cultivars were studied by Ardekani *et al.* (2014). Alkaloids, widely existing in natural plants, are compounds containing basic nitrogen atoms. Most of alkaloids are pharmacologically active ingredients in many medicinal plants due to their significant physiological activity. Many alkaloids can be extracted from natural plant materials and purified by modern separation techniques (Xu *et al.,* 2009). Numerous phytochemical constituents have been reported to be present in different parts of the pomegranate plant making it pharmacologically precious (Prakash and Prakash, 2011).

In general, the potent antioxidant activity of pomegranate is attributed to its polyphenols. Pomegranate polyphenols include flavonoids (flavonols, anthocyanins etc.) as well as condensed tannins (proanthocyanidins), and hydrolyzable tannins (ellagitannins and gallotannins), all of which are substances able to inactivate the products of the oxidative catabolism that trigger cell disorders, aging as well as numerous cardiovascular diseases. Other phytochemicals identified from the pomegranate are organic and phenolic acids, sterols and triterpenoids, fatty acids, triglycerides, and alkaloids. The antioxidant

activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and metal chelators. In fact, over the past few years, many researchers have found that products derived from pomegranate can be used for the prevention and treatment of certain types of cancers such as colon and prostate cancer. Punicalin, punicalagine, granatine β, gallagyldilaclenecasuarine, pedunculagine and tellimagrandine were isolated from the pericarp the fruits also contain punicalagine, punicaline and grantine B. The bark contains iso-pelletierine, pseudo-pelletierine, methyl iso-pelletierine and pelletierine. Similarly, the pomegranate-induced inhibition of the proliferation of breast cancer cells has been documented. The polyphenols contained in the juice, especially anthocyanins and tannins are also capable to quench the effects of ultraviolet (UV) rays, contributing to minimize the primary risk factor of skin cancer. The purpose of the present investigation was to extract and preliminarily characterize the hydrophilic substances from seed oil of pomegranates grown in the Mediterranean region of Italy and to evaluate their potential effects on several colon (HT29 and HCT116), liver (HepG2 and Huh7), breast (MCF-7 and MDA-MB-231) and prostate (DU145) cancer lines (Costantini *et al.,* 2014).

Alkaloids, widely existing in natural plants, are compounds containing basic nitrogen atoms. Most of alkaloids are pharmacologically active ingredients in many medicinal plants due to their significant physiological activity. Many alkaloids can be extracted from natural plant materials and purified by modern separation techniques (Xiao-long *et al*., 2009). Pelletierine is a liquid alkaloid obtained from the root bark of *Punica granatum* Linn. It is an antihelmintic and amoeboid. Pelletierine triggers, like strychnine, a raised stimulant reflex, which can escalate to tetanus, and it is effective against diverse tapeworms, ring worms and nematodes (El-Sakka 2010)**.** Pelletierine is said to occur in the bark of *Punica granatum* together with pseudopelletierine, isopelletierine and methyl pelletierine. It is evident that pelletierine has antihelmintic properties and that the antihelmintic activity of the mixture of alkaloids from *Punica granatum* is mainly (or considerable extent) due to the presence of pelletierine in these mixtures Solvent organic extracts contain a mixture of secondary metabolites including alkaloids, flavonoids, terpenoids, and other phenolic compounds; these molecules are associated with the defense mechanisms of plants by their repellent or attractive properties, protection against biotic and abiotic stresses, and maintenance of structural integrity of plants (Al-Hemiri *et al*., 2009). Previous studies reported that phytochemicals have been identified from various parts of the pomegranate tree and from pomegranate fruit: peel, juice and seeds (Singh *et al.,* 2002; Elfalleh *et al.,* 2009). Therefore, the chemistry and biology of phytochemicals are of highest importance for evaluation of their potential health benefits to humans.

Phenolic compounds, including flavonoids, anthocyanins and tannins, are the main group of antioxidant phytochemicals with interesting properties and have deeply value due to their biological and free radical scavenging activities (Elfalleh *et al.,* 2011). Various parts of *Punica granatum* have been used for various medicinal purposes. Many studies have shown that the pomegranate peel extract possesses wound healing properties (Chidambara *et al.,* 2002), Polyphenol components found in all fruits and vegetables play a major role in many biological activities like colour, flavor, texture as well as antioxidant and antibacterial activities (Negi and Jayaprakash, 2003).The phytochemistry of pomegranate has also been widely studied by some researchers and this fruit is found to be a rich source of polyphenolic compounds (Dandekar *et al*., 2008). Both flavonoids and tannins are more abundant in the peels (El-falleh *et al.,* 2012).

Antioxidant activity of *Punica granatum*

Antioxidants are those substances which possess free radical chain reaction breaking properties. Recently, there has been an upsurge of interest in the therapeutic potential medicinal plants as antioxidants in re-antioxidants in reducing oxidative stress-induced tissue injury (Pourmorad *et al.,* 2006). They are known to inhibit lipid peroxidation (by inactivating lipoxygenase), to scavenge free radicals and active oxygen species by propagating a reaction cycle and to chelate heavy metal ions (Sudarajan *et al.,* 2006). Punicalagins have been identified as the primary components responsible for the reduction of oxidative stress, due to their potent free-radical scavenging ability, which led to these risk factors (Aviram *et al.,* 2000), also, some studies have been carried out in order to investigate the antibacterial effects of extracts against dental plaque (Menezes *et al.,* 2006). Some investigations focus on the toxicity evaluation of whole fruit hydroalcoholic extract of *Punica granatum* L. used in Cuban traditional medicine for the treatment of respiratory diseases; and it was found that toxic effects of *Punica granatum* fruit extract occurred at higher doses than those that are effective in chick embryo models (Vidal *et al.,* 2003).

Phenolics compounds constitute one of the major groups of molecules acting as primary antioxidants or free radical terminators; therefore the total amount of these compounds was determined in the selected pomegranate extracts. Flavonoids, one of the most diverse and widespread group of phytochemical components, are probably the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities, including radical scavenging properties. Such properties are especially distinct for flavonols, such as punicalagins that are found in pomegranate seed pulp (Singh, 2002). Oxidative stress is initiated by free radicals, which seek stability

through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation. These changes contribute to cancer, atherosclerosis, cardiovascular diseases ageing and anti-inflammatory diseases. All human cells protect themselves against free radical damage by enzymes such as superoxide dismutase [SOD] and catalase orcompounds such as ascorbic acid, tocopherol and glutathione. Antioxidant activity has been proposed to play vital role in various pharmacological activities such as anti-aging, anti-inflammatory and anti-atherosclerosis activities (Lee *et al.,* 2005). Inhibition of free radical induced damage by supplementation of antioxidants has become an attractive therapeutic strategy for reducing the risk of diseases. Several synthetic antioxidants are available, but are quite unsafe and their toxicity is of concern (Brash and Harve, 2002). Natural products with antioxidant activity may be used for human consumption because of their safety. Recently there has been an upsurge of interest in the therapeutic potential medicinal plants as antioxidants in re-antioxidants in reducing oxidative stress-induced tissue injury (Pourmorad *et al.,* 2006). Sudarajan *et al*. (2006) studied to inhibit lipid peroxidation (by inactivating lipoxygenase), to scavenge free radicals and active oxygen species by propagating a reaction cycle and to chelate heavy metal ions. There is increasing epidemiological and pharmacological evidence that plants contain biologically active components (e.g. free radical scavengers) offering health benefits and protection against degenerative diseases (Huang and Prior, 2005). Unstable reactive oxygen species (ROS) react rapidly and destructively with biomolecules such as protein, lipid, DNA and RNA in the body. Uncontrolled generation of free radicals is associated with lipid and protein peroxidation, resulting in cell structural damage, tissue injury or gene mutation (Li *et al.,* 2006).

In Biochemistry and Medicine, antioxidants are enzymes or other organic substances, such as vitamin E or β-carotene, that are capable of counter-acting the damaging effects of oxidation in animal tissues and food. It was stated that besides their endogenous defenses, the consumption of dietary antioxidants, such as phenolic compounds, play a vital role in protecting against ROS (Han *et al.,* 2008). In this regard, numerous natural medicinal plants have been evaluated for their antioxidant activities and research outcomes have shown that crude extracts or purified constituents from different medicinal plants were more effective antioxidants *in vitro* than some synthetic antioxidants. Recently, the interest in the antioxidant properties of phenolic constituents from pomegranate fruits (*i.e*., arils and peels) has emerged (Madrigal-Carballo *et al.,* 2009). The phenolic constituents, ellagic tannins and ellagic acid, are among the potent antioxidants in peels (Mutahar *et al.,* 2012). The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants.

Antioxidants, both exogenous and endogenous, whether synthetic or natural, can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders (Heinecke *et al.,* 2003). The antioxidant activity of these plant extracts against linoleic acid, peroxidation and radical scavenging activity against 2,2-diphenyl-1-picrylhdydrazyl (DPPH) were studied in that report. Trolox and quercetin were used as antioxidant reference compounds (Souri *et al*., 2008). Prakash and Prakash (2011**)** studied pomegranate mediated antioxidant activity can be considered a means of lowering the threshold for inflammation. Antioxidant activity, as well as suppression of inflammation, may contribute to chemotherapeutic and chemopreventive utility against cancer.

Antimicrobial activity of *Punica granatum*

The widespread use of commercially available antimicrobials led to the consequence of emergence of antimicrobial resistant pathogens that ultimately led to the threat to global public health. Since 1980s, the introduction of new antimicrobials has declined due to the huge expense of developing and testing new drugs. All commercially available antibiotics with prolonged use may have negative effect on human health because they kill gut flora, so human beings need to take probiotics to replace the killed gut flora. All the above points make a clear way for herbal antimicrobials. The use of plants for treating diseases is as old as the human civilization. There are many plants which have been in use as traditional medicine, so they are called as medicinal plants. The use of plants for curing diseases was inevitable as is already proven by seeing the problems associated with synthetic antibiotics. Peels of some plants as *Punica granatum* which are generally treated as wastes are true antibiotics as they are available for no cost, have no side effects and the most important benefit is that antibiotic resistant pathogens will be easily killed by these new and natural antimicrobials because they will take at least a few decades to get mutated and resistant to them (Khan and Hanee, 2011).

Natural products have been evaluated as sources of antimicrobial agents with efficacies against a variety of microorganisms. Dahham *et al.* (2010) evaluated the antibacterial and antifungal activities of pomegranate peel extract (rind), seed extract, juice and whole fruit on the selected bacteria and fungi. The peel extract has shown highest antimicrobial activity compared to other extracts. Among the selected bacterial and fungal cultures, the highest antibacterial activity was recorded against *Staphylococcus aureus* and among fungi high activity against *Aspergillus niger* was recorded. Gram-positive cocci, and particularly *Staphylococcus* sp., are predominant among the organisms that are responsible for infective complications which have

contributed significantly to the morbidity and mortality of hospitalized patients.

Most staphylococcus infections result in acute diseases. Resistant bacteria representing a challenge in the treatments of various well-known infections necessitated the need to find new substances with antimicrobial properties to be used in the combat against these microorganisms. The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms. Pai *et al*. (2011) investigated the antibacterial activity of Pomegranate rind extracts (alcoholic and aqueous) against various enteric pathogens. Both Standard strains and clinical isolates of *Vibrio cholerae,* Enterotoxigenic *E. coli, Entero pathogenic E. coli, Entero aggregative E. coli, Salmonella and Shigella* species along with few strains of Candida were used in the study. The results obtained were encouraging as the ethanolic extract showed greater zones of inhibition against the various enteric pathogens tested in comparison with the aqueous extract. Most significant inhibitory effect was seen against *Shigella flexneri and Aeromonas hydrophila.* The activities observed could be due to the presence of some of the secondary metabolites like, alkaloids, anthraquinones, sterols, glycosides, saponins, terpenes and flavonoids detected in the plant (Egharevba *et al.,* 2010).

Tayel and El-Tras (2010) screened the antibacterial activity of pomegranate peel extract against *Staphylococcus aureus*, *Bacillus subtillis, Escherichia coli,* and *Pseudomonas aeruginosa* and found significant inhibition compared to other investigators. In a study by Bhadbhade *et al*. (2011), ethanol and water extracts of pomegranate demonstrated inhibitory effects against *S. mutans* and *P. gingivalis*. Further, the results of a Brazilian study suggested that the application of a pomegranate gel may prevent attachment of bacteria in the oral cavity. In other *in vitro* studies, pomegranate extract also inhibited strains of the periodontal bacteria, *P. gingivalis, E. coli* and *Proteus* sp. Chaitra *et al.* (2012) investigated on antimicrobial properties of leaf extracts of *Punica granatum* L. The methanolic extract inhibited *Staphylococcus aureus*, *Bacillus cereus, Salmonella typhi and Proteus mirabilis,* whereas, the chloroform, ethyl acetate and aqueous extracts exhibited moderate inhibitory effect against the test bacteria. On the other hand, only methanolic extract demonstrated antifungal activity against *Aspergillus niger, A. flavus, Trichophyton rubrum, Candida albicans* and *Cryptococcus* sp. Additionally, this plant is reported to have excellent antibacterial, antifungal, antiprotozoal and antioxidant properties. Ashok Kumar and Vijayalakshmi (2013) evaluated *in vitro* antimicrobial activity against bacterial strains *Staphylococcus aureus, Bacillus circulans, Klebsiella pneumoniae, Vibrio vulnificus* and *Salmonella typhi*. The fungal strains selected for the studies were *Candida albicans, Cryptococcus neoformans and Candida tropicalis.*

Punica granatum peel extracts showed higher antimicrobial potential, *Staphylococcus aureus* growth was completely inhibited at lesser concentration by the extracts. Nitave *et al*. (2014) evaluated the antibacterial and antifungal activity of ethanolic extract of *Punica granatum* peel on selected bacterial gram positive and gram negative and fungal cultures. Antimicrobial activity was tested against one gram positive bacteria and three gram negative bacteria while antifungal activity was tested against two fungi. This study represented that ethanol extracts of waste material (peel) of *Punica granatum* may be utilized as a potential source of antimicrobial and antifungal agents. Hijoori *et al*. (2014) evaluations were based on the zone of inhibition using Agar well diffusion assay. The inhibitory activity was found to be dose dependent. The maximum antimicrobial activity was reported at 10 mg/mL dosage tested. Results showed that *Punica granatum* showed highly significant antimicrobial activity against both the classes of bacteria. Aqueous, ethanol and methanol extracts were found to be more active towards the microorganisms tested than acetic acid and petroleum ether extracts. However with respect to pathogens the effectiveness will vary depending on the nature of extraction as well as on concentration of extract being administered. *Salmonella typhi* and *Proteus vulgaris* was reported to have significant susceptibility against most of the extract. That study represents that aqueous, ethanol and methanol extracts of waste material (peel) of *Punica granatum* may be utilized as a potential source of antimicrobial agents. Phytochemical analysis of *P. granatum* peel showed the presence of alkaloids, flavanoids, steroids, tannin, cardiac glycosides and terpenoids using different solvent system suggest extractions of bioactive compounds are solvent dependent. Parashar *et al.* (2014) evaluated the antimicrobial activity of various extracts prepared from pomegranate fruit peels were evaluated using both *in vitro* agar diffusion and *in situ* methods against some food-borne pathogens. It was found that 80% methanolic extract of peels was a potent inhibitor for *Yersinia enterocolitica, Listeria monocytogenes, Staphyllococcus aureus* and *Escherichia coli*.

Conclusion

Pomegranate has been known to be a reservoir of secondary metabolites which are being exploited as source of bioactive substance for various pharmacological purposes. Pomegranate are endowed with various phytochemical molecules such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity. Studies have shown that many of these antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, anti-tumor, anti-mutagenic, anti-carcinogenic, antibacterial, and antiviral activities.

The ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing and in recent years, there has been a worldwide trend towards the use of the natural phytochemicals. In coming years, the secondary metabolites from pomegranate with unknown pharmacological activities may be extensively investigated as a source of medicinal agents.

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